

We claim:

10/522341

- 5 1. A process for preparing transformed plant cells or organisms, which comprises the following steps:
 - 10 a) transforming a population of plant cells, with the cells of said population containing at least one marker protein capable of causing directly or indirectly a toxic effect for said population, with at least one nucleic acid sequence to be inserted in combination with at least one compound capable of reducing the expression, amount, activity and/or function of at least one marker protein, and
 - 15 b) selecting transformed plant cells whose genome contains said nucleic acid sequence and which have a growth advantage over nontransformed cells, due to the action of said compound, from said population of plant cells, the selection being carried out under conditions under which the marker protein can exert its toxic effect on the non-transformed cells.
- 25 2. The process as claimed in claim 1, wherein the marker protein is capable of converting directly or indirectly a substance X which is nontoxic for said population of plant cells into a substance Y which is toxic for said population, which process comprises the following steps:
 - 30 a) transforming the population of plant cells with at least one nucleic acid sequence to be inserted in combination with at least one compound capable of reducing the expression, amount, activity and/or function of at least one marker protein, and
 - 35 b) treating said population of plant cells with the substance X at a concentration which causes a toxic effect for nontransformed cells, due to the conversion by the marker protein, and
 - 40 c) selecting transformed plant cells whose genome contains said nucleic acid sequence and which have a growth advantage over nontransformed cells, due to the action of said compound, from said population of plant cells, the selection being carried out under conditions under which the marker protein can exert its toxic effect on the non-
 - 45

transformed cells.

3. The process as claimed in claim 2, wherein the nontoxic substance X is a substance which does not naturally occur in plant cells or organisms or occurs naturally therein only at a concentration which can essentially not cause any toxic effect.
4. The process as claimed in claim 2 or 3, wherein the substance X is a substance selected from the group consisting of pro-herbicides, proantibiotics, nucleoside analogs, 5-fluorocytosine, auxinamide compounds, naphthalacetamide, dihaloalkanes, Acyclovir, Ganciclovir, 1,2-deoxy-2-fluoro- β -D-arabinofuranosil-5-iodouracil, 6-thioxanthine, allopurinol, 6-methylpurine deoxyribonucleoside, 4-aminopyrazolopyrimidine, 2-amino-4-methoxybutanoic acid, 5-(trifluoromethyl)thioribose and allyl alcohol.
5. The process as claimed in any of claims 1 to 4, wherein the marker protein is selected from the group consisting of cytosine deaminases, cytochrome P-450 enzymes, indoleacetic acid hydrolases, haloalkane dehalogenases, thymidine kinases, guanine phosphoribosyl transferases, hypoxanthine phosphoribosyl transferases, xanthine guanine phosphoribosyl transferases, purine nucleoside phosphorylases, phosphonate monoester hydrolases, indoleacetamide synthases, indoleacetamide hydrolases, adenine phosphoribosyl transferases, methoxinine dehydrogenases, rhizobitoxin synthases, 5-methylthioribose kinases and alcohol dehydrogenases.
6. The process as claimed in any of claims 1 to 5, wherein the marker protein is encoded by
 - a) a sequence described by the GenBank accession number S56903, M32238, NC003308, AE009419, AB016260, NC002147, M26950, J02224, V00470, V00467, U10247, M13422, X00221, M60917, U44852, M61151, AF039169, AB025110, AF212863, AC079674, X77943, M12196, AF172282, X04049 or AF253472
 - b) a sequence according to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46 or 48.
7. The process as claimed in any of claims 1 to 6, wherein the compound capable of reducing the expression, amount, activity and/or function of at least one marker protein comprises at

least one nucleic acid sequence, ribonucleic acid sequence, double-stranded ribonucleic acid sequence, antisense ribonucleic acid sequence, expression cassette or polypeptide sequence.

5

8. The process as claimed in any of claims 1 to 7, wherein the compound capable of reducing the expression, amount, activity and/or function of at least one marker protein is a DNA construct which comprises

10

a) at least one expression cassette suitable for expressing a ribonucleic acid sequence and/or, if appropriate, a protein, said nucleic acid sequence and/or protein being capable of reducing the expression, amount, activity and/or function of the marker protein, or

15

b) at least one sequence which causes a partial or complete deletion or inversion of the sequence coding for said marker protein and thus enables the expression, amount, activity and/or function of the marker protein to be reduced, and also, if appropriate, further functional elements which facilitate and/or promote said deletion or inversion, or

20

c) at least one sequence which causes an insertion into the sequence coding for the marker protein and thus enables the expression, amount, activity and/or function of the marker protein to be reduced, and also, if appropriate, further functional elements which facilitate and/or promote said insertion.

25

9. The process as claimed in any of claims 1 to 8, wherein the expression, amount, activity and/or function of the marker protein are reduced by at least one of the following processes:

30

a) introducing at least one double-stranded marker protein ribonucleic acid sequence or an expression cassette or expression cassettes ensuring expression thereof.

35

b) Introducing at least one marker protein antisense ribonucleic acid sequences or an expression cassette ensuring expression thereof.

40

c) Introducing at least one marker protein antisense ribonucleic acid sequence combined with a ribozyme or an ex-

45

pression cassette or expression cassettes ensuring expression thereof.

- 5 d) Introducing at least one marker protein sense ribonucleic acid sequences for inducing cosuppression or an expression cassette ensuring expression thereof.
- 10 e) Introducing at least one DNA- or protein-binding factor against a marker protein gene, marker protein RNA or marker protein or an expression cassette ensuring expression thereof.
- 15 f) Introducing at least one viral nucleic acid sequence causing degradation of the marker protein RNA or an expression cassette ensuring expression thereof.
- 20 g) Introducing at least one construct for generating an insertion, deletion, inversion or mutation in a marker protein gene.
- 25 10. The process as claimed in any of claims 1 to 9, wherein the marker protein gene is inactivated by introducing a sequence-specific recombinase.
- 30 11. The process as claimed in any of claims 1 to 9, wherein the marker protein gene is inactivated by introducing a sequence-specific enzyme suitable for inducing DNA double-strand breaks.
- 35 12. The process as claimed in any of claims 1 to 11, wherein the marker protein gene is inactivated by inducing an intramolecular or intermolecular homologous recombination.
- 40 13. The process as claimed in claim 12, wherein the homologous recombination is promoted by the action of a sequence-specific enzyme suitable for inducing DNA double-strand breaks.
- 45 14. The process as claimed in any of claims 1 to 13, wherein the marker protein is inactivated by integrating a DNA sequence into a marker protein gene, which process comprises the following steps:
- i) introducing an insertion construct and at least one enzyme suitable for inducing DNA double-strand breaks at a recognition sequence for targeted induction of DNA

double-strand breaks in or close to the marker protein gene, and

- 5 ii) inducing DNA double-strand breaks at the recognition sequences for targeted induction of DNA double-strand breaks in or close to the marker protein gene, and
- 10 iii) inserting the insertion construct into the marker protein gene, with the functionality of the marker protein gene and, preferably, the functionality of the recognition sequence for targeted induction of DNA double-strand breaks being inactivated so that the enzyme suitable for induction of DNA double-strand breaks can no longer cut said recognition sequence, and
- 15 iv) selecting plants or plant cells in which the insertion construct has been inserted into the marker protein gene.
- 20 15. The process as claimed in any of claims 11, 13 and 14, in which the sequence-specific enzyme suitable for induction of DNA double-strand breaks is a Homing endonuclease.
- 25 16. The process as claimed in any of claims 1 to 15, wherein a sequence coding for a resistance to at least one toxin, antibiotic or herbicide is introduced together with the nucleic acid sequence to be inserted and selection is carried out additionally using the toxin, antibiotic or herbicide.
- 30 17. The process as claimed in any of claims 1 to 16, wherein the nucleic acid sequence to be inserted into the genome of the plant cell or of the plant organism comprises at least one expression cassette capable of expressing, under the control of a promoter functional in plant cells or in plant organisms, an RNA and/or a protein which does not cause the expression, amount, activity and/or function of a marker protein to be reduced.
- 35 18. The process as claimed in any of claims 1 to 17, wherein the plant cell is part of a plant organism or of a tissue, part, organ, cell culture or propagation material derived therefrom.
- 40 19. The process as claimed in any of claims 1 to 18 for preparing transformed plant cells or organisms, which comprises the following steps:
- 45

- 5 a) transforming a population of plant cells which comprises at least one non-endogenous (preferably non-plant) marker protein capable of converting directly or indirectly a substance X which is nontoxic for said population of plant cells into a substance Y which is toxic for said population, with at least one nucleic acid sequence to be inserted in combination with at least one nucleic acid sequence coding for a ribonucleic acid sequence capable of reducing the expression, amount, activity and/or function of said marker protein, and
- 10
- 15 b) treating said population of plant cells with the substance X at a concentration which causes a toxic effect for nontransformed cells, due to the conversion by the marker protein, and
- 20 c) selecting transformed plant cells (and/or populations of plant cells, such as plant tissues or plants) whose genome contains said nucleic acid sequence and which have a growth advantage over nontransformed cells, due to the action of said compound, from said population of plant cells, the selection being carried out under conditions under which the marker protein can exert its toxic effect on the nontransformed cells, and
- 25
- 30 d) regenerating fertile plants, and
- 35 e) eliminating by crossing the nucleic acid sequence coding for the marker protein and isolating fertile plants whose genome contains said nucleic acid sequence but does not contain any longer the sequence coding for the marker protein.
- 40 20. An amino acid sequence coding for a plant 5-methylthioribose kinase, wherein said amino acid sequence contains at least one sequence selected from the group consisting of SEQ ID NO: 60, 62, 64, 66 or 68.
- 45 21. A nucleic acid sequence coding for a plant 5-methylthioribose kinase, wherein said nucleic acid sequence contains at least one sequence selected from the group consisting of SEQ ID NO: 59, 61, 63, 65 or 67.
22. A double-stranded RNA molecule, comprising

- a) a "sense" RNA strand comprising at least one ribonucleotide sequence which is essentially identical to at least a part of the "sense" RNA transcript of a nucleic acid sequence coding for a marker protein, and
- 5 b) an "antisense" RNA strand which is essentially, preferably fully, complementary to the RNA sense strand under a).
- 10 23. The double-stranded RNA molecule as claimed in claim 22, wherein the marker protein is defined as in any of claims 2 to 6.
- 15 24. The double-stranded RNA molecule as claimed in either of claims 22 and 23, wherein the "sense" RNA strand and the "antisense" RNA strand are covalently linked to one another in the form of an inverted repeat.
- 20 25. A transgenic expression cassette, comprising a nucleic acid sequence which codes for a double-stranded RNA molecule as claimed in any of claims 22 to 24 and which is functionally linked to a promoter functional in plant organisms.
- 25 26. A transgenic expression cassette, comprising a nucleic acid sequence which codes for at least part of a marker protein and which is functionally linked in antisense orientation to a promoter functional in plant organisms.
- 30 27. The transgenic expression cassette as claimed in claim 26, wherein the marker protein is defined as in any of claims 2 to 6.
- 35 28. A transgenic vector, comprising a transgenic expression cassette as claimed in any of claims 25 to 27.
- 40 29. A transgenic plant organism, comprising a double-stranded RNA molecule as claimed in any of claims 22 to 24, a transgenic expression cassette as claimed in any of claims 25 to 27 or a transgenic vector as claimed in claim 28.
- 45 30. The transgenic plant organism as claimed in claim 29, selected from the group of plants, consisting of wheat, oats, millet, barley, rye, corn, rice, buckwheat, sorghum, triticale, spelt, linseed, sugar cane, oilseed rape, cress, arabis, cabbage species, soybean, alfalfa, pea, bean plants,

peanut, potato, tobacco, tomato, eggplant, paprika, sunflower, tagetes, lettuce, calendula, melon, pumpkin and zucchini.

- 5 31. A tissue, an organ, a part, a cell, a cell culture or propagation material, derived from a transgenic plant organism as claimed in either of claims 29 and 30.

10

15

20

25

30

35

40

45